

In Vivo Glucocorticoid Modulation of Guinea Pig Splenic Macrophage Fc γ Receptors

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Abstract

Although glucocorticoids are widely used in the treatment of immunohematologic disease, their relative efficacy is uncertain. We used an animal model, which has helped to elucidate the role of splenic macrophage Fc γ receptors in the clearance of IgG-coated cells, to investigate whether each Fc γ receptor is modulated by glucocorticoids to the same extent and to examine the relative potency of three commonly used glucocorticoids. Cortisol, prednisone, and dexamethasone all impaired the clearance of IgG-coated erythrocytes. However, dexamethasone was more effective than either prednisone or cortisol ($P < 0.001$). Furthermore, splenic macrophages isolated from glucocorticoid-treated animals expressed impaired Fc γ receptor function. This effect was greater in macrophages isolated from dexamethasone-treated animals, as compared to either cortisol- or prednisone-treated animals ($P < 0.001$). To assess the effect of glucocorticoids on the two types of guinea pig splenic macrophage Fc γ receptors, Fc γ R1,2 and Fc γ R2, specific immunoglobulin isotypes were used to measure macrophage binding of IgG-sensitized erythrocytes. Cortisol and prednisone primarily affected Fc γ R2, whereas dexamethasone inhibited the function of both guinea pig Fc γ receptors. Furthermore, dexamethasone was more effective ($P < 0.01$) than either prednisone or cortisol in inhibiting the ability of both receptors to bind IgG-sensitized cells. Fluorescence-activated cell sorter analysis and fluorescence microscopy with monoclonal antibodies specific for each of these two receptors demonstrated that essentially all splenic macrophages expressed both receptors, and that these glucocorticoids decreased the level of each Fc γ receptor protein expressed, rather than altering receptor mobility and clustering in the macrophage membrane. The effect on both Fc γ receptors was greatest with dexamethasone and least with cortisol. These studies demonstrate the significant role of guinea pig splenic macrophage Fc γ R2 in immune clearance and in the binding of IgG-coated cells. They demonstrate a differential effect of glucocorticoid hormones on Fc γ receptor function and on surface receptor protein. Furthermore, they suggest that dexamethasone may be a more effective glucocorticoid than either prednisone or cortisol in inhibiting the clearance of IgG-

coated cells by its effect on splenic macrophage Fc γ receptors. (*J. Clin. Invest.* 1991. 88:149-157.) Key words: Fc fragment of IgG • glucocorticoid • immune clearance • receptors

Introduction

Receptors for the Fc portion of IgG (Fc γ receptors) are involved in a wide range of physiologic and pathophysiologic processes (1, 2). They are important in the recognition by macrophages of IgG-sensitized cells and are involved in the clearance of immune complexes and bacterial pathogens (3). In autoimmune hemolytic anemia and immune thrombocytopenic purpura (ITP),¹ for example, Fc γ receptors play a central role in the immune clearance of IgG-sensitized erythrocytes and platelets, respectively (4).

Glucocorticoids can substantially alter the course of immunohematologic disease (5). One proposed mechanism for the efficacy of glucocorticoids in these disorders is the impairment of macrophage recognition of IgG-sensitized cells or IgG-containing immune complexes (2, 6). Prednisone is the most widely used glucocorticoid in human immunohematologic disorders, including immune hemolytic anemia and ITP (6). However, the relative potency in vivo of other glucocorticoids, such as dexamethasone, on the macrophage Fc γ receptors is unknown. We used an experimental model developed in the guinea pig to compare the effect of glucocorticoids on one aspect of the pathologic process in immune hemolytic anemia and ITP: splenic macrophage clearance of the IgG-coated cells. We previously have found this model helpful in elucidating the effect of cortisol and other steroid hormones on macrophage clearance of antibody and complement-coated erythrocytes (7-10).

Recently, it has been observed that on macrophages Fc γ receptors are biochemically heterogeneous (1-3). The function of these different Fc γ receptors is as yet uncertain. In the guinea pig two macrophage Fc γ receptors have been identified. Fc γ R2 binds IgG2 and Fc γ R1,2 binds both IgG1 and IgG2 (11). Using this animal model, we assessed the differential effect of the in vivo administration of three commonly used glucocorticoids, cortisol, prednisone, and dexamethasone, on these splenic macrophage Fc γ receptors. Our data indicate that the clearance of IgG-coated erythrocytes is impaired by each of these glucocorticoids through an effect at the level of expression of splenic macrophage Fc γ receptors. The relative potency of the glucocorticoids in this system was dexamethasone > prednisone > cortisol. A differential effect of these steroid hormones on Fc γ receptor function and expression was observed.

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1. Abbreviations used in this paper: ITP, immune thrombocytopenic purpura; MFI, mean fluorescence intensity; SSV, steroid suspension vehicle.

Methods

All studies were performed with male Hartley guinea pigs weighing 500–600 g obtained from Dutchland Farms, Denver, PA. Glucocorticoids were obtained from Steraloids, Inc., Wilton, NH. All animals were injected with an equal volume of a homogeneous suspension of glucocorticoids in a vehicle (SSV) consisting of 0.5% carboxymethylcellulose, 0.4% Tween 80, and 1.5% ethanol in isotonic saline. This suspension vehicle has been used in our laboratory and the laboratories of others for subcutaneous injection to examine the biologic effects of steroids (9, 10, 12). Sham-treated animals received 1 ml of SSV alone, and the drug-treated animals received glucocorticoids suspended in SSV, injected subcutaneously in the dorsal neck fat pad every morning for 7 d. Each animal was studied on the day after the seventh injection. IgG anti-guinea pig erythrocyte antibody was prepared in rabbits and quantitated as previously described (7). The IgG fraction was isolated by Sephadex S-300 chromatography and anion exchange chromatography, and was free of IgM as determined by Ouchterlony analysis and SDS-PAGE.

Clearance of IgG-coated erythrocytes. Blood was drawn from guinea pigs by cardiac puncture. Washed erythrocytes were radiolabeled with ^{51}Cr -sodium chromate (New England Nuclear, Boston, MA) and sensitized with an equal volume of IgG antibody, so as to be coated with $\sim 3,000$ IgG molecules per erythrocyte (7, 13). At least 1,500 IgG molecules per erythrocyte were necessary to accelerate clearance, similar to our previous observations (7, 13). The number of IgG molecules per erythrocyte was determined using ^{125}I -labeled IgG as described (7, 14). Animals pretreated with glucocorticoids or SSV control for 7 d were injected intravenously with 1.7×10^8 ^{51}Cr -labeled cells. Samples of blood were obtained from the retroorbital space 1–120 min after injection and cell-associated radioactivity was measured in a γ -counter (Gamma 8000, Beckman Instruments, Inc., Fullerton, CA). Studies were also performed with heat-altered erythrocytes to investigate splenic clearance mediated by nonimmune mechanisms (9, 10). To determine the organ responsible for the clearance of sensitized and heat-altered erythrocytes, guinea pigs were killed after the clearance studies. Their livers, kidneys, lungs, and spleen were removed, and whole-organ ^{51}Cr radioactivity was determined.

Clearance curves were plotted by expressing the number of blood counts per minute at each time point as a percentage of the number of counts per minute at 5 min. Clearance at time points 60, 90, and 120 min was analyzed to calculate a P value for the difference between control and experimental clearance curves using a Student's t test. In addition, for each day's clearance study, the percent inhibition of clearance (mean \pm SEM) above control was calculated at 90 and 120 min according to the formula: percent inhibition = $100 \times [(c_{\text{pm}_c} - c_{\text{pm}_x}) / (c_{\text{pm}_c} - c_{\text{pm}_{\text{ea}}})]$, where c_{pm_c} refers to counts per minute for the untreated control animal injected with unsensitized cells, c_{pm_x} to the experimental animal treated with glucocorticoid and injected with IgG-coated erythrocytes, and $c_{\text{pm}_{\text{ea}}}$ to control animals treated with SSV only (no glucocorticoid) and injected with IgG-sensitized erythrocytes. A negative value for percent inhibition indicates enhancement of clearance. This formula compares treated animals with the control animals studied on the same experimental day, and expresses the data as percent alteration of clearance, where 100% inhibition of clearance by glucocorticoids corresponds to the situation in which the clearance of IgG-sensitized erythrocytes (c_{pm_x}) is identical to that of unsensitized erythrocytes (c_{pm_c}) (9, 10).

Binding of IgG-coated erythrocytes by splenic macrophages in vitro. Guinea pigs were killed, splenectomy was performed immediately, and the spleens were placed in RPMI 1640 + 10% heat-inactivated fetal calf serum + glutamine (complete RPMI). Fine suspensions of spleen cells in PBS with no Ca^{++} or Mg^{++} were obtained using tissue grinding sieves with a 100- μm mesh, followed by Nytex gauze sieving (50 μm , Tetko Inc., Elmsford, NY). The cells were then layered onto a discontinuous Percoll (Pharmacia, Inc., Piscataway, NJ) gradient (35–70%) and spun at 600 g and 22°C for 30 min. Mononuclear cells were recovered from the intermediate layer (45–55% Percoll). The cells were then washed at

room temperature with PBS containing 5 mM EDTA + 10% heat-inactivated FCS previously dialyzed with PBS without Ca^{++} or Mg^{++} , and resuspended to 2×10^6 cells/ml in complete RPMI. Cells were incubated for 60 min at 37°C in a 5% CO_2 atmosphere in 75-cm 2 tissue culture flasks, to allow the macrophages to adhere. Nonadherent cells were removed with complete RPMI, and adherent cells were mechanically dislodged after 10 min of incubation at 37°C in a 5% CO_2 atmosphere with PBS containing 5 mM EDTA + 10% dialyzed heat-inactivated FCS. Cells were washed once and resuspended in complete RPMI. More than 95% of the resultant cells were viable mononuclear cells as determined by their ability to exclude trypan blue, and > 90% of cells ingested latex beads and were stained with nonspecific esterase. Monolayers of adherent cells were prepared as previously described by incubating 1×10^6 cells on a glass coverslip in a 35-mm plastic petri dish at 37°C for 45 min under 5% CO_2 (9). More than 95% of the cells were adherent to glass.

For experiments studying Fc γ receptor activity in vitro, guinea pig erythrocytes were coated with 800 molecules of IgG per erythrocyte as described above, and 1 ml of erythrocytes (5×10^7 cells/ml) was incubated with the macrophage monolayers at 37°C under 5% CO_2 for 20 min. The monolayers were washed, air-dried and stained with Wright-Giemsa, and 200 consecutive macrophages were inspected under oil immersion for the number of erythrocytes bound per cell (15). The number of macrophages which bound three or more erythrocytes was then determined.

Production and isolation of guinea pig IgG1 and IgG2 anti-sheep erythrocyte antibodies. Two receptors for IgG have been described on guinea pig macrophages, Fc γ R2, which only binds monomeric and aggregated IgG2, and Fc γ R1,2, which binds both monomeric and aggregated IgG1 and IgG2 (11, 16). The ability of soluble guinea pig IgG1 and IgG2 to inhibit the binding of IgG-coated erythrocytes by splenic macrophages in vitro was studied, in order to assess the relative effect of glucocorticoids on each Fc γ receptor. Two types of IgG-sensitized erythrocytes were used: (a) guinea pig erythrocytes sensitized with ~ 800 molecules of rabbit IgG per cell and (b) sheep erythrocytes sensitized with ~ 600 molecules per cell of either purified guinea pig IgG1 or IgG2 anti-sheep erythrocyte antibody.

To prepare isotype-specific antibodies, guinea pigs were immunized with sheep erythrocytes (17). IgG anti-sheep erythrocyte antibody was isolated from the sera of the immunized animals and control animals by ammonium sulfate precipitation, quaternary aminoethyl ion exchange chromatography, and S-300 gel filtration (18). IgG1 and IgG2 anti-sheep erythrocyte antibodies were separated on DEAE-cellulose columns, as previously described (19–21). No IgG isotype contamination was detectable by double immunodiffusion.

Inhibition of binding of IgG-coated erythrocytes by splenic macrophages in vitro. 0.5 ml of guinea pig IgG1 or IgG2 without anti-guinea pig and anti-sheep erythrocyte antibody activity, (0.5–80 $\mu\text{g}/\text{ml}$) was added to the macrophage monolayers for 10 min at 37°C under 5% CO_2 . Then, 0.5 ml of either rabbit IgG-coated guinea pig erythrocytes (5×10^7 cells), guinea pig IgG1-sensitized sheep erythrocytes (1×10^8 cells), or guinea pig IgG2-sensitized sheep erythrocytes (1×10^8 cells) were added to the macrophage monolayers and the assay was continued as above. After incubation, the monolayers were washed and the percentage of macrophages having three or more erythrocytes bound was determined (10).

Flow cytometry. Monoclonal antibodies with specificity against guinea pig macrophage Fc γ R1,2 (VIA2 IgG1) and Fc γ R2 (VIA1 IgG1) (16) were utilized in indirect immunofluorescence binding studies to assess Fc γ receptor protein expression. These monoclonal antibodies were the generous gift of Drs. Yamashita and Nakamura, Sapporo, Japan. Cells (5×10^5) were incubated with saturating concentrations of each monoclonal antibody for 60 min at 4°C and washed twice with phosphate-buffered saline (PBS) containing 0.5% bovine serum albumin and 0.02% sodium azide. To measure bound antibody, a FITC-labeled goat anti-mouse antibody (Tago, Inc., Burlingame, CA) was added for 30 min at 4°C. The cells were again washed twice and fixed with 4% paraformaldehyde. Cell-associated fluorescence was

measured using a FACSTAR cytometer with Consort-30 software (Becton, Dickinson & Co., Mountain View, CA). For all samples, 10,000 events were recorded on a logarithmic fluorescence scale, and the actual mean fluorescence intensity (MFI) for each sample was determined using the Consort-30 software. In order to correct for autofluorescence, the MFI of a nonreactive murine IgG1 antibody (P3) was subtracted from the MFI of the anti-Fc γ R1,2- and anti-Fc γ R2-stained cells. Percent change in fluorescence intensity was calculated by:

$$\% \text{ change} = \left[\frac{(\text{MFI of anti-Fc}\gamma\text{R-treated cells} - \text{MFI of P3-treated cells})}{(\text{MFI of anti-Fc}\gamma\text{R-untreated cells} - \text{MFI of P3-untreated cells})} \right]^{-1} \times 100.$$

Effect of in vivo glucocorticoids on membrane mobility of Fc γ R1,2 and Fc γ R2. Immunofluorescent capping experiments were performed in order to examine any possible effects of in vivo administered steroids on the membrane mobility of Fc γ R1,2 and Fc γ R2. Splenic macrophages (5×10^5) from guinea pigs treated with 25 mg/kg dexamethasone for 7 d, or from sham-treated animals, were incubated with saturating concentrations of monoclonal antibodies for 30 min at 0°C on ice. After two washes at 0°C in PBS/0.5% BSA without sodium azide, FITC-labeled goat anti-mouse antibody was added as in the flow cytometry experiments. Cells were incubated at either 0°C or 37°C for 20 min, washed, fixed in paraformaldehyde, and spun onto microscope slides in a centrifuge (Cytospin, Shandon, Inc., Pittsburgh, PA). Several hundred cells per slide were examined under epifluorescence with a fluorescent microscope (Carl Zeiss, Oberkochen, Germany).

Results

Cortisol pretreatment of guinea pigs impaired splenic clearance of IgG-coated erythrocytes (Fig. 1). In agreement with previous studies, pretreatment for 7 d with 90 mg/kg of cortisol impaired the clearance of IgG-sensitized erythrocytes (RBCs) in 22 of 25 animals by $42 \pm 3\%$ compared with simultaneous sham-treated controls ($P < 0.001$, Fig. 1) (8, 9). The lowest effective dose of cortisol which inhibited the clearance of IgG-sensitized RBCs was 10 mg/kg ($P < 0.01$, Fig. 1).

Prednisone given for 7 d at doses of 90, 25, 5, and 1 mg/kg to groups of 25 animals also impaired the clearance of IgG-coated erythrocytes in a dose-dependent fashion. In 3 of 25 animals, prednisone pretreatment (90 mg/kg) had no effect on clearance. In the remaining 22 animals, clearance was inhibited by $78 \pm 6\%$. Animals pretreated with 25 mg/kg of prednisone exhibited a $58 \pm 4\%$ inhibition of clearance ($P < 0.001$, Fig. 1). The lowest dose of prednisone that reproducibly inhibited the clearance of IgG-coated RBCs was 1 mg/kg ($P < 0.01$, Fig. 1). Thus, prednisone inhibited macrophage Fc γ receptor-dependent clearance at lower doses of injected drug than did cortisol.

Pretreatment of 29 of 30 animals for 7 d with 90 mg/kg of dexamethasone impaired the clearance of IgG-coated RBCs by $78 \pm 5\%$, compared with sham-treated animals studied in parallel ($P < 0.001$, Fig. 1). This effect also depended upon the dose of dexamethasone administered over a range of 0.05–90 mg/kg. The percentages of cells remaining in the circulation at 2 h ranged from $78 \pm 5\%$ (90 mg/kg) to $31 \pm 4\%$ (0.05 mg/kg, $P < 0.01$), respectively, compared with $21 \pm 4\%$ for the sham-treated controls (Fig. 1). Thus, dexamethasone demonstrated greater inhibitory activity at equimolar concentrations than either cortisol or prednisone.

The effects of dexamethasone, prednisone, and cortisol in vivo were compared by determining the dosage required to

impair clearance by 50% (IC_{50}) (Fig. 2). The IC_{50} for dexamethasone (4 mg/kg) was ~ 3 -fold lower than that for prednisone (13 mg/kg) and > 18 -fold lower than that for cortisol (74 mg/kg).

To assess the effect of glucocorticoids on Fc γ receptor-independent clearance, we compared the RBC survival of heat-altered erythrocytes in glucocorticoid-treated and sham-treated animals. The percentage of cells in the circulation fell progressively over 2 h and the rate of clearance was dependent on the length of time the cells were heated. ^{51}Cr quantitation in whole spleen, liver, kidneys, and lungs indicated that clearance also took place predominantly in the spleen. Glucocorticoids did not substantially decrease the splenic clearance of heat-altered erythrocytes, as they did the clearance of IgG-sensitized erythrocytes (Fig. 3).

We then studied the effect of these in vivo glucocorticoids on macrophage Fc γ receptor expression assessed in vitro. Splenic macrophages were isolated from animals after treatment in vivo with glucocorticoids, and their in vitro binding of IgG-coated erythrocytes examined (Fig. 4). 60% of the isolated splenic macrophages from sham-treated controls bound at least three IgG-coated RBCs. Pretreatment with glucocorticoids decreased macrophage binding of the IgG-sensitized erythrocytes in a dose-dependent manner. Dexamethasone was significantly ($P < 0.001$) more effective than prednisone and cortisol in inhibiting splenic macrophage Fc γ receptor-dependent binding of IgG-coated cells in vitro. When inhibition of splenic macrophage binding of IgG-sensitized RBCs was assessed by linear regression analysis, the IC_{50} for dexamethasone (3 mg/kg) was ~ 20 -fold lower than the IC_{50} for prednisone and > 60 -fold lower than the IC_{50} for cortisol (Fig. 5). Thus, the relative potency of dexamethasone compared with prednisone and cortisol in inhibiting in vitro splenic macrophage binding of IgG-coated cells appears more than that predicted by its relative anti-inflammatory potency.

Two Fc γ receptors have been described on guinea pig peritoneal macrophages (11, 16). We determined that guinea pig splenic macrophages also express Fc γ R1,2 and Fc γ R2 (see below). Both receptors bind guinea pig IgG2, while only Fc γ R1,2 binds IgG1. Thus, the effect on Fc γ R1,2 can be selectively examined by studying the interaction with IgG1. Animals were pretreated with 25 mg/kg of glucocorticoid, splenic macrophages were isolated, and binding of rabbit IgG-coated RBCs was examined in the presence or absence of purified guinea pig IgG1 or IgG2 (Table I). We calculated the concentration of guinea pig IgG1 and IgG2 that inhibited binding of the IgG-coated cells by 50%. The IC_{50} for IgG1 did not significantly differ among splenic macrophages isolated from sham-treated animals or animals treated with prednisone or cortisol. However, the IC_{50} for IgG2 was significantly decreased by all three glucocorticoids. Thus, these glucocorticoids appear to primarily affect binding to guinea pig splenic macrophage Fc γ R2. The IC_{50} for dexamethasone-treated animals was substantially lower ($P < 0.01$) than that for prednisone and cortisol. In addition, dexamethasone inhibited both guinea pig splenic macrophage Fc γ receptors, Fc γ R1,2 and Fc γ R2.

Sheep erythrocytes selectively sensitized with either guinea pig IgG1 or guinea pig IgG2 anti-sheep RBC antibody were also prepared. Table II illustrates the inhibition of splenic macrophage binding of these subclass-specific antibody sensitized RBCs by soluble guinea pig IgG1 or IgG2. IgG1 inhibited splenic macrophage binding of IgG1-sensitized RBCs to the same extent whether the cells were isolated from prednisone- or

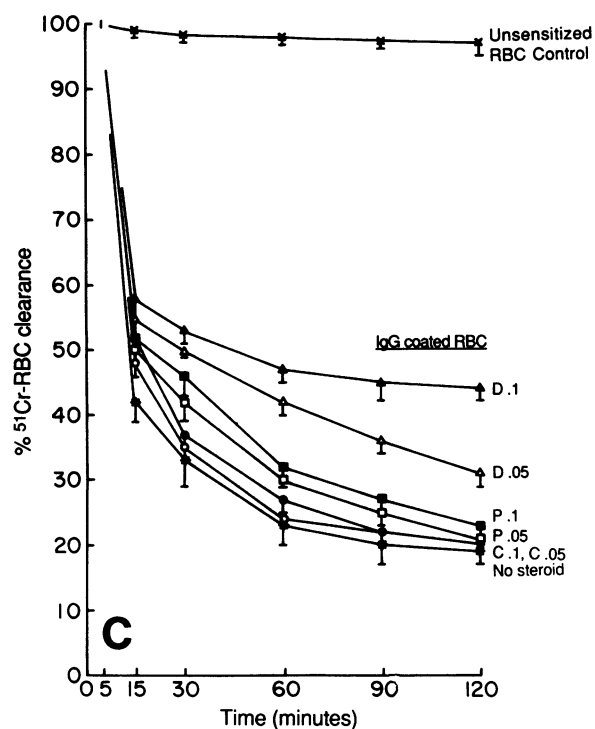
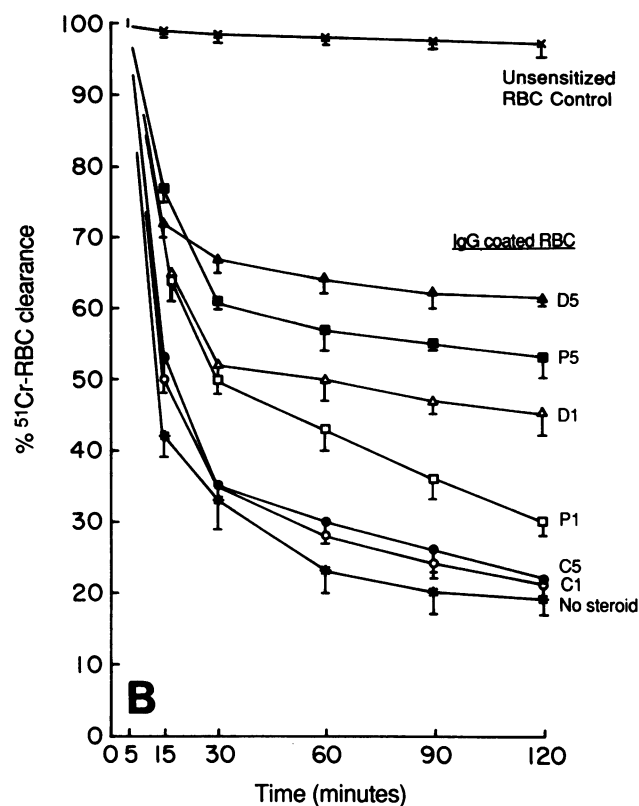
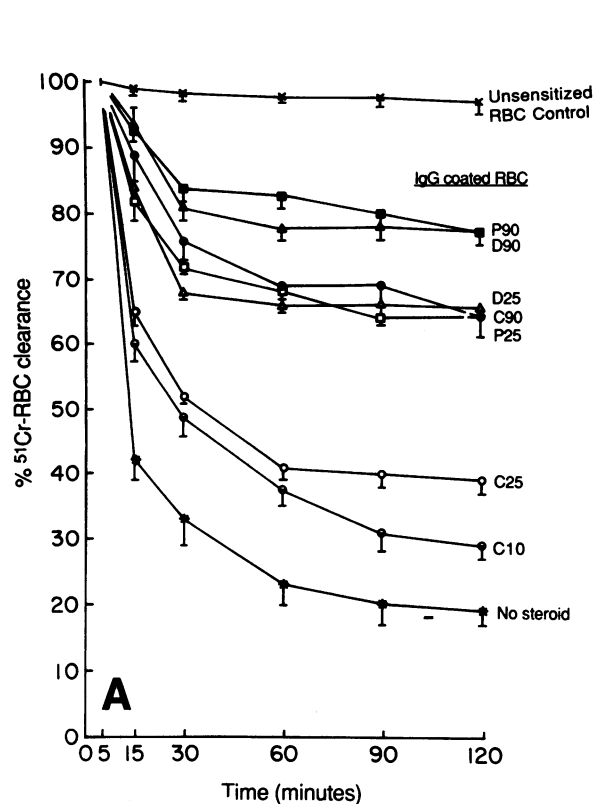


Figure 1. Clearance of IgG-coated erythrocytes (RBC) in guinea pigs pre-treated with glucocorticoids (0.05–90 mg/kg) for 7 d. The dose-dependent effect of either cortisol (C), prednisone (P), or dexamethasone (D) is shown and compared with sham-treated controls. The results with different dosages of glucocorticoid are shown in A, B, and C. The percent ^{51}Cr -RBC clearance represents the percentage of remaining circulating RBCs at each indicated time point.

cortisol-treated animals or from sham-treated animals. However, IgG1 was more effective when the macrophages had been isolated from dexamethasone-treated animals. Lower concentrations of IgG2 were effective at inhibiting the binding of IgG2-sensitized RBCs when macrophages derived from ani-

mals treated with any of the three glucocorticoids were compared to sham-treated controls. These data imply that cortisol and prednisone appear to primarily affect binding to $\text{Fc}\gamma\text{R2}$, whereas dexamethasone inhibits the function of both guinea pig $\text{Fc}\gamma$ receptors, $\text{Fc}\gamma\text{R1,2}$ and $\text{Fc}\gamma\text{R2}$. Furthermore, dexamethasone

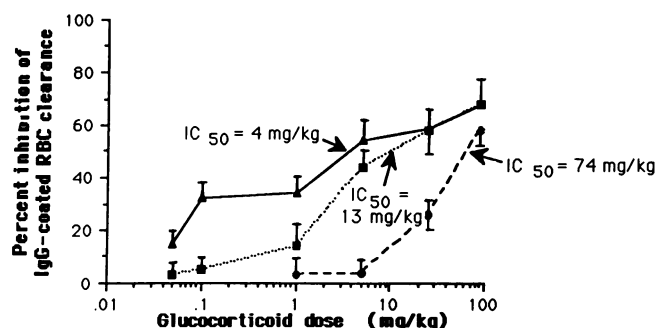


Figure 2. Inhibition of clearance of IgG-coated erythrocytes by glucocorticoids. The glucocorticoid dose inhibiting the clearance of IgG-coated cells by 50% (IC_{50}) is shown for dexamethasone (▲), prednisone (■), and cortisol (●).

methasone was more effective ($P < 0.01$) than either prednisone or cortisol in inhibiting the ability of both receptors to bind IgG-sensitized cells.

To determine whether these functional changes reflected changes in the actual level of each type of $Fc\gamma$ receptor expressed on the cell surface, macrophage $Fc\gamma$ receptors were also studied by FACS analysis, using specific monoclonal antibodies against each of these receptors. Fluorescence histograms are shown in Fig. 6. Virtually all of the guinea pig splenic macrophages expressed each $Fc\gamma$ receptor ($Fc\gamma R1,2 > 95\%$ and $Fc\gamma R2 > 85\%$). Animals pretreated for 7 d with 90 and 50

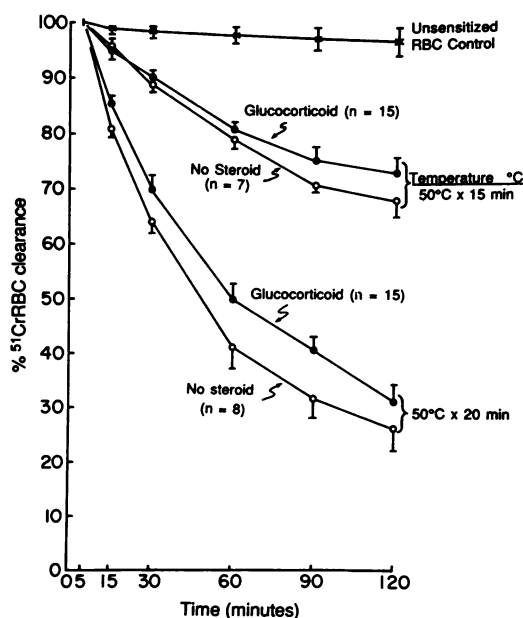


Figure 3. Clearance of heat-altered erythrocytes. Erythrocytes were heated to 50°C for 15 or 20 min and injected into glucocorticoid-treated animals (90 mg/kg of either cortisol, prednisone, or dexamethasone for 7 d) or sham-treated controls. No selective differences in the clearance of heat-altered RBCs were found when animals treated with each glucocorticoid were compared ($P > 0.5$). Thus, a common clearance curve is shown for the three glucocorticoids. The percent ^{51}Cr -RBC clearance represents the percentage of remaining circulating RBCs at each time point.

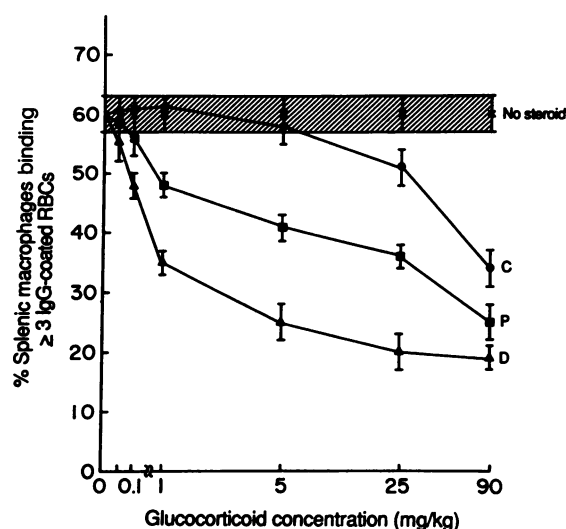


Figure 4. Inhibition of the in vitro binding of IgG-coated erythrocytes by splenic macrophages isolated from guinea pigs pretreated for 7 d with cortisol (C), prednisone (P), or dexamethasone (D) at the indicated glucocorticoid dosages. The in vivo administered dose of glucocorticoid is indicated on the abscissa. The percentage of the splenic macrophages binding more than three IgG-coated RBCs is presented. The results with sham-treated controls (no steroid) are shown by the hatched rectangle.

mg/kg per day of cortisol exhibited a $40 \pm 3\%$ and a $38 \pm 2\%$ decrease in the membrane expression of splenic macrophage $Fc\gamma R1,2$ respectively (Fig. 7). The lowest effective dose of cortisol inducing a significant inhibition in $Fc\gamma R1,2$ expression was 50 mg/kg. Prednisone (20–90 mg/kg) induced a 60–66% decrease in the expression of guinea pig $Fc\gamma R1,2$. No significant alteration in the expression of guinea pig splenic macrophage $Fc\gamma R1,2$ was detected with doses of prednisone under 10 mg/kg per day. Dexamethasone (1–90 mg/kg per day) decreased the membrane expression of $Fc\gamma R1,2$ by 53–86%. The lowest effective daily dose of dexamethasone which impaired the expression of guinea pig splenic macrophage $Fc\gamma R1,2$ was 1 mg/kg (Fig. 7). Therefore, at equimolar administered doses, the effect on $Fc\gamma R1,2$ was greatest with dexamethasone and least with cortisol.

Glucocorticoids also inhibited the level of $Fc\gamma R2$ protein after administration in vivo (Fig. 7). Animals pretreated for 7 d with 20–90 mg/kg per day of cortisol exhibited a 28–35% inhibition of $Fc\gamma R2$ expression. The lowest effective daily dose of cortisol inducing an effect was 10 mg/kg. Prednisone at doses of 10–90 mg/kg induced a 30–57% decrease in $Fc\gamma R2$ expression. Finally, dexamethasone (1–90 mg/kg) decreased the membrane expression of guinea pig splenic macrophage $Fc\gamma R2$ by 48–84% (Fig. 7). Inhibition of both $Fc\gamma$ receptors by dexamethasone (1 mg/kg) required 3 d of treatment and persisted until 2 d after administration (data not shown). These data indicate that in vivo glucocorticoids inhibit the expression of guinea pig splenic macrophage $Fc\gamma$ receptor protein, and that among these three glucocorticoids dexamethasone is the most potent.

We considered whether the highly lipophilic glucocorticoid molecules might alter the mobility of surface membrane receptors, thus contributing to the inhibitory effects observed for

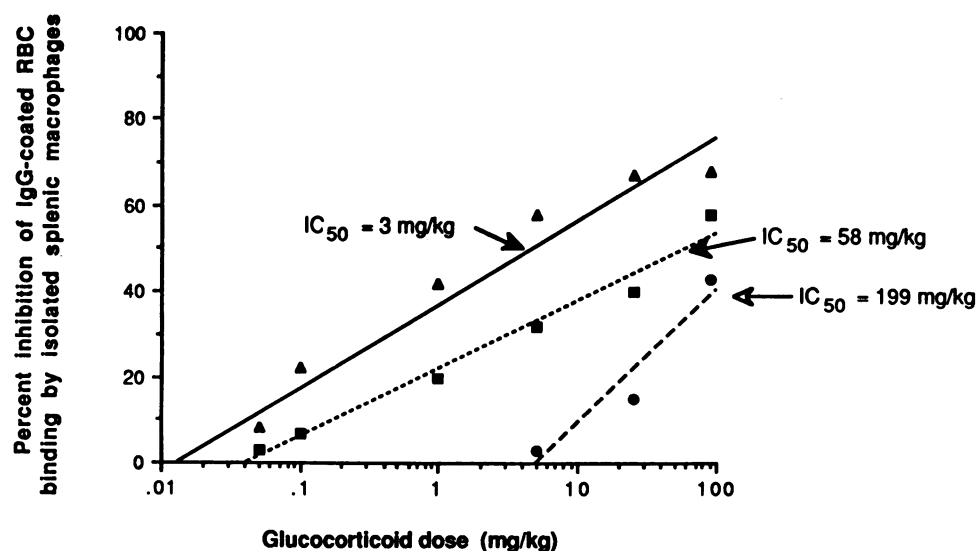


Figure 5. Inhibition of the in vitro binding of IgG-coated erythrocytes by glucocorticoids. The in vivo glucocorticoid dose inhibiting 50% of the binding of IgG-coated RBCs by splenic macrophages (IC_{50}) is shown. The IC_{50} for dexamethasone (3 mg/kg) was 19- and 66-fold lower than that for prednisone (58 mg/kg) and cortisol (199 mg/kg), respectively.

$Fc\gamma R1,2$ and $Fc\gamma R2$ function. To this end, in vitro capping experiments were performed comparing splenic macrophages isolated from treated guinea pigs to those from sham-treated animals. A relatively high dose of dexamethasone (25 mg/kg), which was found to be the most potent agent in studies described above, was chosen. Cells incubated at 0°C to prevent membrane movement showed a uniform diffuse (ring) pattern, when stained for either $Fc\gamma R1,2$ or $Fc\gamma R2$. When incubated at 37°C, the majority of cells displayed aggregates or patches of membrane fluorescence, with some cells showing an intense polar distribution of staining for both $Fc\gamma$ receptors, similar to that reported for the ligand-induced capping of lymphocyte surface immunoglobulin (22). No significant differences were observed between sham or 7-d dexamethasone-treated animals for either $Fc\gamma R1,2$ or $Fc\gamma R2$ staining intensity or distribution. Therefore, it appears that glucocorticoids do not have a major effect on the membrane mobility of these receptors, suggesting that their inhibitory effects are likely at the level of surface protein expression.

Discussion

It has been established using monoclonal antibodies that there are at least two biochemically distinct $Fc\gamma$ receptor proteins in

$Fc\gamma R1,2$ and $Fc\gamma R2$, have been shown to be present on lymphocytes and peritoneal macrophages. Our data suggest that these receptors are also expressed on essentially all splenic macrophages. Furthermore, when the macrophages from the spleen are isolated, each of these $Fc\gamma$ receptors participates in the binding of IgG-sensitized erythrocytes.

However, it is uncertain whether the signals that govern the expression of each of these receptors are the same. For example, there may be differences in steroid-sensitive regulatory elements in each $Fc\gamma$ receptor gene. To investigate whether each receptor is modulated by glucocorticoids to the same extent and to examine the relative potency of several commonly used glucocorticoids, we employed an established experimental model of splenic macrophage $Fc\gamma$ receptor-mediated immune clearance.

Prednisone is the most widely used glucocorticoid in the treatment of immunohematologic diseases, such as IgG-induced autoimmune hemolytic anemia and ITP. Its effect on the splenic clearance of IgG-coated erythrocytes has not been previously compared with that of cortisol and dexamethasone. In fact, although it had been demonstrated that cortisol impairs the clearance of IgG-coated cells (8–10), the effect of prednisone and dexamethasone on immune clearance has not been

Table I. Inhibition by Monomeric Guinea Pig Subclass-specific IgG of Splenic Macrophage Binding of Rabbit IgG-coated Erythrocytes

IC_{50}	Sham	Cortisol*	Prednisone	Dexamethasone
IgG1 ($\mu g/ml$)	160	154 (4%)	148 (7.5%)	44 (72.5%)
IgG2 ($\mu g/ml$)	64	30 (53%)	23 (64%)	7 (89%)

* Animals were pretreated for 7 d with each glucocorticoid at a dose of 25 mg/kg. The percent inhibition, compared to sham controls, of macrophage binding of IgG-sensitized RBCs is indicated in parentheses.

Table II. Inhibition by Monomeric Guinea Pig Subclass-specific IgG of Splenic Macrophage Binding of Guinea Pig IgG1 or IgG2-sensitized Erythrocytes (RBCs): Effect of Glucocorticoids (25 mg/kg)

IgG	Sham	Cortisol	Prednisone	Dexamethasone
IgG1-RBC				
IC_{50} ($\mu g/ml$ IgG1)	72	70 (3%)	68 (6%)	18 (75%)
IgG2-RBC				
IC_{50} ($\mu g/ml$ IgG2)	48	31 (35%)	25 (48%)	11 (77%)

The percent inhibition, compared to sham controls, of macrophage binding of IgG-sensitized RBCs is indicated in parentheses.

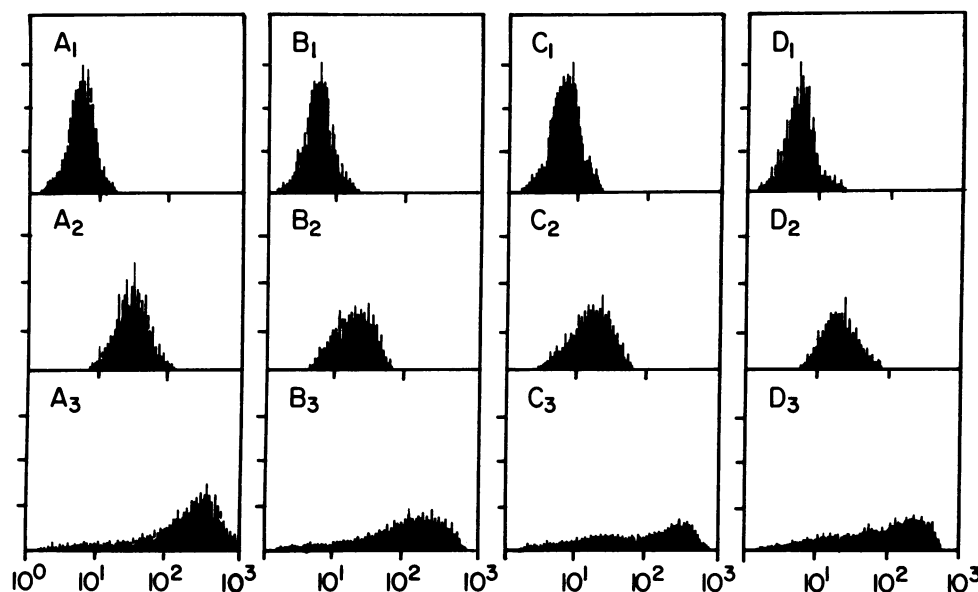


Figure 6. Cytofluorographic histograms of guinea pig splenic macrophages from (A) untreated animals and (B) animals treated with dexamethasone (1 mg/kg per d), (C) prednisone (20 mg/kg per d), or (D) cortisol (50 mg/kg per d). Cells were stained with (1) isotype control, (2) anti-Fc γ R1,2, or (3) anti-Fc γ R2.

established. In our study, treatment with cortisol, prednisone, and dexamethasone inhibited splenic macrophage Fc γ receptor recognition of IgG-coated cells *in vitro* and *in vivo*. Furthermore, similar to the therapeutic experience in man, we observed that the majority of, but not all animals respond to glucocorticoids. The reason for this heterogeneity is uncertain,

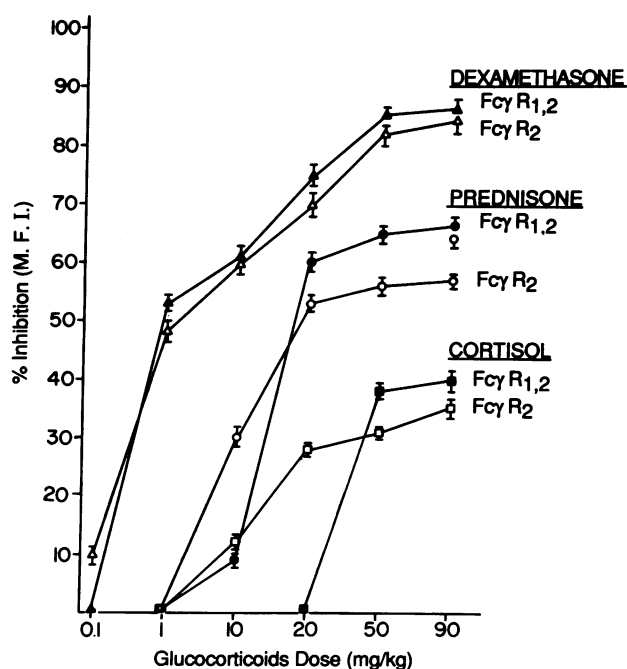


Figure 7. Expression of Fc γ R1,2 (solid symbols) and Fc γ R2 (open symbols) on isolated guinea pig splenic macrophage after treatment for 7 d with dexamethasone (triangles), prednisone (circles), or cortisol (squares). The daily *in vivo* administered dose of each glucocorticoid is indicated on the abscissa. Changes (percent inhibition) in Fc γ receptor expression are plotted as the mean percent change in the mean fluorescence intensity (M.F.I.) of cells from treated animals.

but could involve genetic differences in the extent to which the glucocorticoids are activated or metabolized or differences in macrophage receptor expression itself.

In both the *in vitro* and *in vivo* systems, the apparent rank order of potency of the glucocorticoids was dexamethasone > prednisone > cortisol. FACS analysis of the isolated splenic macrophages revealed a similar pattern of potency for the steroid effect on Fc γ receptor expression. These data suggest that dexamethasone may be more effective than either prednisone or cortisol in the therapy of such disorders as IgG-induced immune hemolytic anemia or ITP in which macrophage Fc γ receptors are important in the clearance of these immune complexes. Our data suggest that somewhat lower doses of dexamethasone than are required for its anti-inflammatory action (24) may be effective in these immunohematologic diseases.

We also employed FACS analysis to study the mechanism of the glucocorticoid effect. It had been uncertain whether glucocorticoids inhibited Fc γ receptor function, e.g., alteration of their affinity or mobility in the membrane, or whether glucocorticoids decreased Fc γ receptor number. Our experiments indicate that glucocorticoids decrease the number of both Fc γ R1,2 and Fc γ R2 on the cell surface. This suggests that glucocorticoids can down-regulate Fc γ receptor expression. In experiments to directly address the effect of glucocorticoids on receptor membrane mobility, we used dexamethasone at a dosage that markedly inhibited immune complex clearance and the binding of IgG-sensitized RBCs. The observation that receptor patching and capping, induced by Fc γ receptor cross-linking, was unaffected for both Fc γ R1,2 and Fc γ R2 is consistent with the interpretation that the primary effect of glucocorticoids is on Fc γ receptor number.

We used two approaches to assess which of the macrophage Fc γ receptors is affected by glucocorticoids. First, we used the observation that guinea pig macrophage Fc γ R1,2 is selectively inhibited by guinea pig IgG1 to assess Fc γ R1,2 function *in vitro*. The data suggest that Fc γ R2 is more affected than Fc γ R1,2 by these steroid hormones (Tables I and II). Furthermore, since cortisol and prednisone inhibit clearance at dos-

ages that only affect Fc γ R2, the data also suggest that splenic macrophage Fc γ R2 plays a major role in the clearance of IgG-sensitized cells.

Second, we used FACS analysis with monoclonal antibodies specific for guinea pig macrophage Fc γ R1,2 and Fc γ R2. We observed a dose-dependent modulation by glucocorticoids of macrophage Fc γ R1,2 and Fc γ R2. Although these data are consistent with a predominant effect on Fc γ R2, we observed that in vivo cortisol and prednisone similarly inhibit Fc γ R1,2 and Fc γ R2 surface expression (Fig. 7) at dosages that alter the in vitro binding of IgG2 but not IgG1 (Tables I and II). Since IgG2 interacts with both Fc γ R1,2 and Fc γ R2, the decreased surface expression of Fc γ R1,2 may contribute to the decreased binding of IgG2. One possibility for these differences is that under these conditions the Fc γ R1,2 binding site for IgG1 is still expressed. More likely, these observations may be explained by differences in affinity of these receptors for their respective ligands, e.g., differential affinity of Fc γ R1,2 for guinea pig IgG1 and IgG2. For example, if IgG1 binds with high affinity to Fc γ R1,2, then no inhibition of IgG1 binding might be observed even with substantial reduction in Fc γ R1,2 expression. Alternatively, it is possible that these glucocorticoids alter Fc γ R2 in a manner that diminishes IgG2 binding either through a direct effect on Fc γ R2 or by altering another macrophage membrane protein necessary for Fc γ R2 function.

We have attempted to assess the effect of glucocorticoids on Fc γ R1,2 and Fc γ R2 affinity using soluble monovalent and polyvalent IgG1 and IgG2. However, high levels of nonspecific binding to splenic macrophages have prevented us from making this determination. We have observed in other systems that glucocorticoids in vitro can alter the function of cell surface Fc γ receptors (25). In the latter studies glucocorticoids inhibited the capacity of IgG aggregates to mediate Fc γ receptor-dependent platelet activation without diminishing Fc γ receptor surface expression.

We observed that dexamethasone differs from both cortisol and prednisone not only in its potency, but also in its equivalent inhibition of Fc γ R1,2 and Fc γ R2 surface expression at all doses examined (Fig. 7). Furthermore, functional studies demonstrated both a more potent and an equivalent inhibition of guinea pig IgG1 and IgG2 binding (Tables I and II). These data suggest that dexamethasone is a potent inhibitor of Fc γ receptor function and surface expression and are consistent with dexamethasone's potency in inhibiting immune clearance (Fig. 2).

There is substantial similarity between humans and guinea pigs in their response to glucocorticoids. Both species are steroid resistant and are similar in their steroid metabolism (26, 27). There is also evidence that steroid effects on macrophages are similar (28). We have previously measured the circulating levels of steroid hormones in the guinea pig and observed that they correlate with both the administered in vivo dose and with the steroid levels seen during changes in hormonal state in humans (9, 10). Thus, our data may have relevance to human disease. We have previously observed that steroid hormones can influence human monocyte/macrophage Fc γ receptor expression in vitro and in vivo (2, 15, 29, 30). Furthermore, it appears that some Fc γ receptors, e.g., human Fc γ R1, are more responsive to such modulatory signals than are other macrophage Fc γ receptors (2, 28, 30, 31), similar to the relative preferential effect of glucocorticoids on guinea pig splenic macro-

phage Fc γ R2. However, the precise homology between the guinea pig and human macrophage Fc γ receptors awaits the cloning of the guinea pig Fc γ receptor genes. Similarly, these genes will be necessary in order to determine whether glucocorticoid hormones influence macrophage Fc γ receptor transcription or posttranscriptional events. It is of interest that a recent report of the genomic sequence of a murine Fc γ receptor reveals the presence of a glucocorticoid responsive element in that gene (32).

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